

REMARKS

Each rejection presented in the Office Action mailed March 19, 2004 is discussed below.

Objections to the specification

The designated portion of the specification has been amended for clarification.

35 USC §112 ¶2

Claim 3 has been amended as suggested. The suggestion is appreciated.

Claim 1 has been amended to delete the phrase "functional component" and to replace it with "catalytically active".

Claim 1 has been amended to delete the phrase beginning "whereby".

Claim 1 has been amended to delete the phrase concerning "plaminogen activity".

Claim 1 has been amended to clarify the term "time lag".

Claim 1 has been amended to clarify the preamble phrase "containing essentially".

Claim 1 has been amended to clarify the referenced fibronectin binding domain pairs.

35 USC §112 ¶1

As detailed in the enclosed Declaration of Dr. Girish Sahni, streptokinase is a well-studied protein. The inventive concept is that catalytically active SK can be targeted to clots with fibrin binding domains and that the catalytic activity can be delayed and targeted, thereby enhancing the amount of activity directed to the desired plasmin-rich clot site and avoiding indiscriminant activity elsewhere. Various streptokinases are known, and there is absolutely no issue of: a) determining the minimal fragment required for catalytic activity; and b) using such a fragment in the invention. The use of fibrinectin finger domains is similarly well characterized. Prior art of record also establishes this point.

The Examiner is respectfully requested to review the enclosed declaration and to provide a specific rationale (as opposed to a generalized statement that applies to all proteins) supporting the conclusion that the invention would be read by those skilled in the art as being limited to

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specific parent sequences, and to exclude other well-characterized components of the claimed hybrid that were known in the art.


In particular, the Examiner reviews the inventive concept at page 9, line 12 through page 10, line 7 of the Office Action. Clearly that inventive concept is not limited to any specific parent sequence. it applies generally to fusions within the claim scope. Applicants have not only done experiments with four specific constructions, they have established a plasmin-dependent mechanism that applies to all constructions that include a component with SK activity and a component with fibrin binding domains that will target the SK activity to clots which are plasmin-rich. The Examiner has offered no rationale for the proposed claim limitation, which are simply reflexive, arbitrary sequence limitations that are unjustified in this case. Here, the inventive concept is novel and broadly applicable to an art that was well aware of alternative species of each of the two hybrid components.

The undersigned respectfully requests the favor of a telephone interview prior to any further office action in the event that the Examiner is inclined to maintain this rejection.

Enclosed is a \$950.00 check for the Petition for Extension of Time fee. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: 9/20/04



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Sahni et al.

Serial No.: 09/940,235

Filed: 27th August 2001

*For: Novel clot-specific streptokinase proteins
possessing altered plasminogen activation
characteristics and a process for the preparation of
said proteins*

Group Art Unit: 1652

Examiner: Sheridan L. Swope

Attorney Docket No: 07064-009002

To,
The Assistant Commissioner for Patents
Washington, D.C. 20231

Declaration Under 37 C.F.R. §1.132

I, Girish Sahni, aged 48 years, residing at Chandigarh, INDIA, and a citizen of India, do hereby state as under.

I am a Scientist at the Institute of Microbial Technology, sector 39-A, Chandigarh-160036, India. I graduated in the year 1976 from Panjab University located at Chandigarh, INDIA. I completed my Master's Degree in Microbiology from Panjab University at Chandigarh, INDIA in the year 1978. Subsequently, I completed my doctoral degree in Biochemistry from the Indian Institute of Science, Bangalore, India in the year 1984.

After completing my doctoral degree, I took up my first assignment as a post-doctoral scientist with the University of California, Santa Barbara, USA in the year 1984. After that, I joined the Rockefeller University, New York as senior research associate, and as Adjunct Faculty in 1986. Subsequently, in 1991, I joined as a senior faculty member at the Institute of Microbial Technology (IMTECH), Chandigarh, India, a constituent institution of the Council of Scientific and Industrial Research, India, where I am continuing to now work on molecular biology and structure of proteins for the last twelve years. Presently, I am working as the Deputy Director of this institute (since 2001).

One of the projects undertaken by IMTECH, Chandigarh, INDIA is "*Novel clot-specific streptokinase proteins possessing altered plasminogen activation characteristics and a process for the preparation of said proteins*". This project was undertaken in the year 1992. The scientists involved in the study were myself, Rajesh Kumar, Chaiti Roy, Kammara Rajogopal, Deepak Nihalani, Vasudha Sundaram, and Mahavir Yadav. I am the main scientist (project leader) in this study. I am aware of US patent application No. 09/940,235 filed in respect of this project. I am also aware and familiar with all the office actions, objections of the Examiner and the references cited by the Examiner. Therefore, I am completely and fully aware of all the facts relating to this project as well as the present patent application.

The Examiner has acknowledged enablement limiting to sequences of figures 17b, 19b, 21b, and 22 b. However, an enablement rejection is been set with reasoning set forth in the office action of March 19, 2004 that there is not sufficient substantiation for any hybrid plasminogen activator comprising any streptokinase, or its catalytically active fragment, and any pair of fibrin binding-domains derived from fibronectin, as worded in claims.

I respectfully submit that streptokinase (SK) is a well-studied, rather thoroughly-studied, protein. The structural and functional aspects of the SK are known in the art. Further, I would like to state that the catalytically active fragments within *S. equisimilis* – the prototypic SK source whose SK has been the subject of extensive scientific investigations world-wide in the last thirty years, and which is almost exclusively used as the SK drug source for thrombolytic therapy throughout the medicine world – are also very well known. Other SKs, from various other streptococcal strains have also been extensively studied and found to be structurally closely similar to the SK of *S. equisimilis*. More importantly, even in case of any unknown (new) SK, or a modified SK variant such as that produced by mutagenesis or truncation, technical approaches allowing for the correct and *facile* judgement regarding its suitability for being employed for designing "successful" SK-FBD fusions through the process of the present invention are also well detailed in the art/public domain. In addition, the fibrin binding domains (FBDs) of proteins such as fibronectin, blood coagulation factor/s, tissue plasminogen activator etc belong to a small "family" of 'finger' type FBDs with highly conserved sequence, structure and function that also, similar to the case of the conserved family of streptokinases, would easily allow selection of functionally active domains/derivatives/variants of such domains so as to allow the successful construction of the chimeric proteins disclosed in the present invention. In support of the above, I would like to cite several references, and discuss their salient scientific contributions, which would establish that all structural and functional aspects of SK as well as FBDs are sufficiently well understood for the enablement of the invention, and are in public domain.

In the Public Domain, a rich information base has long been available on the sequence variability, as also the structural and functional properties, of various streptokinases secreted by different streptococcal species. Interestingly, the basic structural 'design' of different streptokinases are very similar to each other (Welfle et al., 1997; *Proteins: Structure, Function, and Genetics* vol. 27, pp. 26-35). The detailed three-dimensional structure of *Streptococcus equisimilis* SK (the protein drug used in human cardiovascular treatment world-wide) in complexation with micro-plasminogen has also been solved (Wang et al., 1998; *Science* vol. 281, pp. 1662-1665), which provides an atomic level high-resolution information base for all future rationale-based protein engineering studies on SK.

From the literature in the public domain (e.g. see: Malke, H., 1993; *Zbl. Bakt.* vol. 278 pp. 246-257) it is now abundantly clear that, overall, only a very limited sequence dissimilarity exists amongst SK proteins secreted from streptococcal strains of different sources such as human, porcine, bovine, canine etc, wherein it is observed that all of the mature SK proteins possess 414 residues, whose overall sequence homologies are very high, in the order of 80-98 percent (Malke, H., 1993; *Zbl. Bakt.* vol. 278 pp. 246-257; see, particularly, page 250, para 1, lines 7-9). In some strains that are associated with nephritic syndromes, the SK proteins are seen to have small regions/loci that have relatively higher internal sequence heterogeneity, but such regions are not present in the SK of strains of human origin used/preferred for SK thrombolytic therapy (Malke, H., 1993; *Zbl. Bakt.* vol. 278 pp. 246-257, and references cited in this scientific publication). Thus, most SK's, irrespective of animal source, are closely homologous to the prototype SK from *S. equisimilis* H46A that, almost exclusively, has been used for human cardiovascular therapy. It also happens to be the most rigorously studied SK till date from the scientific point-of-view, and, therefore, the SK that was chosen to illustrate the present invention.

It is unambiguously clear from the knowledge now available in the public domain that different SK's, because of their close similarity to each other in terms of sequence, function and structural design, can be predicted to display closely similar structure-function co-relationships. It is a well established paradigm in the chemical and biological sciences that similar structures beget similar functional properties. In the biological context, particularly as it relates to the critical issue at hand viz. how facile it will be, or will not be (depending on one's viewpoint), to employ "any" streptokinase, or a modified streptokinase, and use the approaches detailed in the present patent specification to design hybrid fusions of the type disclosed therein, we wish to categorically state that the state-of-the-knowledge in the streptokinase and protein/rDNA engineering fields unambiguously allows a person skilled in these arts to successfully accomplish the objectives employing variant SK and fibrin binding

domain (FBD) sequences with a minimal effort and still obtain the desired functions of fibrin binding together with a plasmin-dependent time-delayed plasminogen activation property.

The Examiner has rejected Claims 1 and 32 on the basis of insufficient enablement since (see page 4, para 2, line 8 onwards of Office Action dated 3.19.2004; Art Unit 1652) "the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired plasminogen activation and fibrin binding requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification) and detailed knowledge of the ways in which the protein's structure relates to its function", and that "in this case the disclosure is limited to the plasminogen activators encoded by the sequences set forth in Figs 17b, 19b, 21b, and 22b."

As explained above, a large knowledge pool already exists on the closely similar structural and functional properties of various SK proteins, a fact that strongly supports the notion that unexpected deviation from the prototype SK used to illustrate the present invention is highly unlikely. It should be appreciated, moreover, that what the present invention 'requires' for successfully designing a functional hybrid, even from an hitherto "unknown/new" SK or FBD fragment, is *not* a pre-knowledge of the "essentially infinite possible choices" of new or mutant sequences but an ability to easily select out ones that are suitable for use in constructing the chimeric/fusion SK-FBD proteins. *The sole criterion for selection, as is abundantly clear from the specification (particularly where the rationale for the designs is explained), is that the selected SK fragments should display a plasminogen activator ability to begin with (that is, prior to fusion), which then becomes reversibly attenuated as a result of the fusion with FBDs.* In the case of *S. equisimilis* SK, the minimal fragment that continues to exhibit such a property is 16-383, a fact that is amply clear from several studies available in the scientific literature (some are cited below to substantiate these conclusions). Remarkably, these detailed studies on SK truncations which have been available in the public domain since several years not only identify the minimal SK region/s consistent with survival of PG activation, but more importantly, provide a very clear-cut description of the experimental approaches/procedures that are easily applicable to the identification of such minimal regions in any given SK/SK mutant, particularly since most SKs are closely related to each other anyway.

Thus, either by truncation of the SK protein by proteolytic enzymes, or by expressing partial-length SK fragments using recombinant DNA techniques, followed either by rapid *in situ*

screening (Malke and Ferretti., 1984; *Proc Nat'l Acad Sci* vol. 81, pp. 3557-3561) for surviving plasminogen activator function in the truncated/expressed fragments or by conventional tube-based assays for PG activator functions (Jackson et al., 1981; *Methods Enzymol* vol. 80, pp. 387-392), the minimal SK fragment length consistent with a given SK, or a SK mutant with Pg activator function can be easily identified, thereby transcending the unnecessary 'limitation' imposed by the requirement of a pre-knowledge of the functional properties of the virtually "infinite possible choices" of variant sequences to ensure success in the design of intended fusions. It may be mentioned that such an empirical approach (essentially screening based) rather than that requiring rational prediction is now a very well-established and accepted procedure in drug discovery/new drug design area provided a facile screening method for a desired property is available. In the case of streptokinase (whose essential functional property is plasminogen activation), a facile and rapid *in situ* screen has been available for a number of years (see: Malke and Ferretti., 1984; *Proc Nat'l Acad Sci* vol. 81, pp. 3557-3561; Saksela, 1981; *Analytical Biochemistry* vol. 111, pp. 276-282). In fact, a multitude of scientific studies have used this approach to study both truncated fragments, as well as site-directed and combinatorial mutants of SK so as to discriminate rapidly between those that are active or inactive for the PG activation capability (see, for example: Lee et al., 1989; *Biochem Biophys Res Comm* vol. 165, pp. 1085-1090; Shi et al., 1994; *Biochem J* vol. 304, pp. 235-241; Young et al., 1995; *J Biol Chem* vol. 270, pp. 29601-29606; Reed et al., 1995; *Biochemistry* vol. 34, pp. 10266-10271; Nihalani and Sahni., 1995; *Biochem Biophys Res Comm* vol. 217, pp. 1245-1254; Lin et al., 1996; *Biochemistry* vol. 35, pp. 16879-16885; Kim et al., 1996; *Biochem Mol Biol Int'l* vol. 40, pp. 939-945; Lee et al., 1997; *Biochem Mol Biol Int'l* vol. 41, pp. 199-207; Fay and Bokka, 1998; *Thromb. Haemost.* Vol. 79, pp. 985-991; Kim et al., 2000; *Thromb Res* vol. 99, pp. 93-98). Many more examples of such scientific studies are available, but only a handful are cited here for brevity.

Thus, sufficient guidance is *already* available to rapidly and easily reject SK-related sequences that are are inactive with respect to PG activation and select out those that are active. These are then to be utilised for the second important step of the enablement, namely fusion with the FBDs derived from the human fibronectin protein. The various fusions, once made, are then screened/selected for the *final desired functions* viz. reversible, plasmin-mediated, time-delayed PG activation as well as fibrin binding as detailed in the patent specification. Thus, enablement of the invention is not just dependent on a "characteristic or properties other than the functionality of encoding a hybrid plasminogen activator having a plasmin-dependent inactivation mechanism that delays plasminogen activation" alone (Cf. Office Action dated 3.19.04 page 8, paragr. 4, line 4) but, as is described above, on selecting out from the myriad possible SK variant/mutant sequences those that are positive for

plasminogen activation to begin with by means that are clearly in the public domain. The same criterion essentially applies for the selection of the FBDs, which have a clear functional property of independent fibrin binding based on their pronounced affinity for fibrin; indeed, detailed procedures for selecting such domains, particularly FBDs 4,5 and 1,2 that have maximal independent affinity amongst all the FBDs of fibronectin, are available in the public domain (see: Rostagno et al., 1994; *J Biol Chem* vol. 269, pp. 31938-31945; Matsuka et al., 1994; *J Biol Chem* vol. 269, pp. 9539-9546). Moreover, the FBDs of fibronectin are structurally and functionally highly conserved structures (e.g. see also: Williams et al., 1993; *Biochemistry* vol. 32, pp. 7388-7395; Potts et al., 1999; *Biochemistry* vol. 38, pp. 8304-8312, and references cited therein). Thus, fibrin affinity function-based procedures are easily applicable for the selection of *any given variant sequences of the FBDs* which can then be taken up for obtaining the fusions between the SK sequence/s selected on the basis of their PG activating ability and the functional FBDs to prepare the chimeric constructs with the desired function of delayed-action PG activation kinetics as well as fibrin binding properties.

In summary, the enablement of our invention is *not solely* dependent on a fore-knowledge of the effect of a given truncation/modification of either the SK or FBD moieties of the hybrids since both can be independently selected by facile assay procedures (based on their respective PG activation and fibrin binding properties) that are available in the public domain. Given the facts that (i) detailed methods exist to produce and test various fragments or derivatives of SK and FBDs (even if not previously tested) for PG activator and fibrin binding functions *independently of each other*, (ii) both the SK and FBD sequences as well as structures are generally highly conserved across species, and (iii) a vast data base of structure-function co-relationship studies on SK mutants that are compatible with the survival of PG activator capability exists, it is completely reasonable to assume that any person skilled in the art can easily select a given SK or FBD for its suitability to be utilised for the design of SK-FBD, FBD-SK or FBD-SK-FBD type of fusion constructs revealed in the patent specification that exhibit a delayed action PG activation kinetics profile together with fibrin binding capabilities – two final properties that can also be easily selected by screening procedures detailed in the specification.

Now, a person who is skilled in this art, is fully aware of not only the functional and structural realities of SK but is also fully aware of the methods and techniques to identify catalytically active fragments within SK. So, it is not at all difficult or intellectually challenging for such a skilled person to replace one SK with another SK or its catalytically active fragments. Now, taking aforementioned aspects into consideration, it is reasonable to state that the hybrids

covered under the amended claims, in response to the Office Action of March 19, 2004 are fully enabled.

Further, I would like to respectfully submit that the invention is limited to hybrid formation between catalytically active fragments of SK and domain 1 and 2 and/or 4 and 5 of fibronectin. As mentioned above, the determination of active fragments is within the range of a skilled person. Also, the domain pairs are well understood in terms of sequential, structural, and functional aspects. Now, binding domains at the N- or C- terminal/s of SK, its active mutants or its known active fragments in not a challenging task for a skilled person. I take this opportunity to inform that we have indeed carried out the fusion of various active fragments of SK as well as active mutants of SK, both those reported in literature as well those generated in our own laboratory, to the fibronectin domains and found the hybrids to be workable. I did not notice any significant structural and functional change in the resultant hybrids from the ones exemplified in the description. We also utilised genes coding for new SKs derived from various hospital isolates of streptococcal species, and after expressing these and selecting for the functionally active fragments, we employed the truncated genes to construct FBD fusions exemplified by the designs disclosed in the patent application, and found these hybrids as well to exhibit the two desired activities viz. delayed, plasmin-dependent activation of PG and strong binding with fibrin. Thus, the description can be easily considered to provide sufficient enablement.

Further, the Examiner must appreciate that it is practically difficult, if not impossible, to incorporate all the possibilities of the active fragments of SK and the hybrid activators in the application. However, here, it can be conveniently assumed that a person skilled in the art would be able to extrapolate the presently available information using conventional methodologies of the art, without incorporating any intellectual input from his side and would still be able to reach/select/express catalytically active fragments of SK and their encoding genes which can then be fused with the fibrin binding domain expressing genes to prepare the hybrids.

Now, taking aforementioned facts into consideration, a skilled person is fully aware that the hybrid formed between FB domains of 1 and 2 and/or domains 4 and 5, and SK or SK's catalytically active fragments would be a well-defined hybrid. There would be nothing ambiguous about it. A person skilled in the art would be able to predict the nature of the

hybrid formed through various possibilities encompassed within the scope of the invention. Further, the nature of the interaction between SK and FBDs is sufficiently elaborated in the specification, especially in Examples 3 to 6. The examples provide the workability model. Now, a skilled person can easily extrapolate the same and reach the other hybrids of the invention, without facing any intellectual challenge.

Thus, I respectfully request the examiner to view the invention in a holistic manner and have a favourable consideration about the enabling aspects of the invention

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Dated: 16.08.2004

Place: Chandigarh, INDIA


GIRISH SAHNI

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